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SYSTEMIC IMMUNE RESPONSE BEFORE AND AFTER TREATMENT OF HELICOBACTER PYLORI INFECTION

Original Article

HELİKOBAKTER PİLORİ ENFEKSİYONUNDA TEDAVİ ÖNCESİ VE SONRASI SİSTEMİK İMMUN CEVAP

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ABSTRACT

Objectives: Although Helicobacter pylori (Hp) infects about 50% of the population, only a small proportion of individuals develop gastritis, peptic ulcer, MALT lymphoma or cancer. This may be due to factors influencing bacterial virulence or the host response to Hp. In this study, we aimed to find out systemic immune response changes in Hp positive peptic ulcer and chronic gastritis before and after eradication treatment.

Methods: Ten patients with Hp associated peptic ulcer or chronic antral gastritis (Group 1), and 10 controls consisted of Hp negative patients with dyspepsia (Group 2) were enrolled to the study. CD3, CD4, CD8, CD19, $CD16^{+}56^{+}$, CD25, and CD54 expression on lymphocytes were evaluated by flow cytometry and cytokines (IL-2, IL-4, IL-10, TGF- β, IFN-γ) were analysed by ELISA in group 1 before and after eradication treatment and in group 2.

Results: Peripheral T-lymphocytes and CD25 expression of Hp(+) patients were significantly increased compared to Hp(-) patients (47.7±5.1% vs 40.6±2.0% respectively, $p<0.001$; 14.4±7.5% vs 5.9±0.7% respectively, $p<0.05$). After eradication treatment circulating T-lymphocytes decreased significantly compared to pretreatment levels (41.9±4.2% vs 47.7±5.1% respectively, $p<0.01$) and the ratio of CD25(+)

lymphocytes decreased down (14,4±7,5% vs 7,7±1,8% respectively, p<0.05).

Conclusions: Although increased T lymphocyte proliferation and activation observed in Hp(+) patients, no change was observed in cytokine levels. Concluding a definite decision about the systemic response to Hp infection is difficult due to these conflicting results. Therefore, more comprehensive studies, including more patients with gastric ulcer, duodenal ulcer, gastritis and/or duodenitis and with a wider panel of immune response measurands, should be performed to clarify this subject.

Key words: *Helicobacter pylori (Hp);cytokines; lymphocyte subtypes.*

ÖZET

Helikobakter pilori dünya nüfusunun %50'sini enfekte etmesine rağmen sadece çok az bir kısmında gastrit, peptik ülser, MALT lenfoma veya kansere yol açmaktadır. Bu sonuç bakteriyel virülans faktörlerine veya konakçının HP'ye karşı olan cevabına bağlı olabilir. Bu çalışmada Hp pozitif gastritli ve peptik ülserli hastalarda eradikasyon tedavisi öncesi ve sonrası sistemik immün cevap değişikliklerini göstermeyi amaçladık. Hp ilişkili peptik ülseri veya kronik antral gastriti olan 10 hasta (grup 1) ve Hp negatif dispepsisi olan 10 hasta (grup2) kontrol grubu olarak seçilmiştir. CD3, CD4, CD8, CD19, CD16+56, CD25 ve CD54 ekspresyonu flow sitometri ile değerlendirilmiş olup sitokinler (IL-2, IL-4, IL-10, TGF-beta, IFN-gama) her iki grupta tedavi öncesi ve sonrası ELİSA ile çalışılmıştır. Hp pozitif hastalardaki periferal T lenfositler ve CD25 ekspresyonu Hp negatif hastalara göre anlamlı olarak artmış bulunmuştur. (sırasıyla %47.7±5.1 ve %40.6±2.0, p<0.001); (sırasıyla %41.9±4.2 ve %47.7±5.1, p<0.01).

Eradikasyon tedavisi sonrası dolaşımdaki T-lenfositleri tedavi öncesi düzeylere kıyasla anlamlı derecede azalmıştır (sırasıyla %41.9±4.2 ve %47.7±5.1, p<0.01) ve CD25 pozitif lenfositlerin oranının da azaldığı tespit edilmiştir (sırasıyla %14.4±7.5 ve %5.9±0.7, p<0.05). Hp pozitif hastalarda T lenfosit proliferasyonu ve aktivasyonu artmış olarak tespit edilmesine karşın sitokin düzeylerinde anlamlı bir farklılık tespit edilememiştir. Bu çelişkili sonuçlar Hp infeksiyonuna karşı gelişen sistemik immün cevap hakkında kesin yargılara varmamızı güçleştirmektedir. Bu konunun açığa kavuşması için daha çok sayıda gastrik ülser, gastrit ve/veya duodeniti olan hasta içeren geniş çalışmaların yapılması gerekmektedir.

Anahtar kelimeler: *Helikobakter pilori;sitokinler;lenfosit subtipleri.*

INTRODUCTION

Helicobacter pylori (Hp) is a gram negative spiral microorganism involved in various gastrointestinal diseases including peptic ulcer, chronic type-B gastritis, gastric carcinoma and MALT lymphoma (1). Hp eradication has become a regular treatment choice in peptic ulcer and chronic type-B gastritis. Although Hp infects about 50% of the population, only a small proportion of individuals develop gastritis, peptic ulcer or cancer (1). This may be due to factors influencing bacterial virulence or host response to Hp. Studies on the pathogenesis of Hp associated gastritis or ulcer is based on the analysis of T-cell proliferation and cytokine production in peripheral blood lymphocytes as indicators of the inflammatory response in infected gastric mucosa (2-9). However, these studies show conflicting results. Some studies suggest that T-cell proliferative responses do not change with Hp infection (5, 6), whereas some other authors claim that T-cell proliferative responses are higher in Hp positive subjects (2, 7). In another study it has been observed that, when peripheral blood lymphocytes were stimulated with Hp antigens T-cell proliferation increased (8). A more recent study claimed that, there is no systemic

alteration in the specific immune system while responding to Hp in patients with duodenal ulcer or chronic antral gastritis (9). In this study, we investigated the changes in systemic immune response in Hp positive peptic ulcer and chronic gastritis before and after eradication treatment.

MATERIALS AND METHODS

Ten patients with Hp associated peptic ulcer or chronic antral gastritis (Group 1), and 10 controls consisting of Hp negative patients with dyspepsia (Group 2) were enrolled to the study (**Table I**).

Group 1: Hp(+) PU or CAG [Rapid Urease Test (+), histology (+)] (n=10)
Group 2: Hp(-) PU and non-CAG with dyspepsia [Rapid Urease Test (-), histology (-)] (n=10)
PU=Peptic Ulcer, CAG=Chronic Antral Gastritis

Table I: *Study groups.*

Informed consent was obtained from all the participants and the study was approved by Ethics Committee of Karadeniz Technical University Medical Faculty. Patients with any other acute or chronic infection/inflammation, experienced gastric resection, bleeding diathesis and patients received antibiotics, non-steroidal anti-inflammatory drugs and proton pump inhibitors within the previous two months were excluded from the study. Each patient was considered Hp positive if the combination of rapid urease test and histological examination were positive for Hp. If both of the test results were negative the patients were accepted as Hp negative. After diagnosis, we treated the Hp positive patients with lansoprasole 30 mg b.i.d., clarithromycin 500 mg b.i.d., and amoxicillin 1 gr b.i.d. After 14 days we cut Clarithromycin and amoxicillin treatment out and continued the medication with lansoprasole for further 4 weeks. Peripheral blood samples for immunologic testing were collected into EDTA containing blood collection tubes (BD Vacutainer Tubes, USA) before treatment. Endoscopic examination was repeated 4 weeks after the completion of drug therapy. Eradication was defined on the basis of both negative urease test and histology.

FLOW-CYTOMETRY

Peripheral blood samples were collected from group 1 and group 2 in EDTA containing tubes (BD Vacutainer Tubes, USA) for flow-cytometric analysis. We added one hundred μl of these samples in 20 μl double-colored monoclonal antibody containing tubes. The incubation of aliquots containing approximately $1x10^6$ mononuclear cells/ml was carried out in dark with fluorescein isothiocyanate (FITC)-labeled monoclonal antibodies for 20 minutes at 4ºC. After staining process, cells were washed with 0.01M phosphate-buffered saline (PBS) pH 7.4 twice and fixed with %4 PFA in PBS and analysed with a flow cytometer (Beckman Coulter Epics XL-MCL, Florida, USA). Monoclonal antibodies were determined as CD3 (PE), CD4 (FITC), CD8 (PE), CD19 (FITC), CD16+56 (PE), CD25 (PE), and CD54 (FITC), purchased from Immunotech, Marseille, France. The same procedure was performed for Group 1 after the Hp eradication was confirmed.

CYTOKINE ANALYSIS

Blood samples collected from the Group 1 and 2 patients at the beginning of the study and the sera were stored at -80°C. The second sample was drawn from the Group 1 patients after the Hp eradication was confirmed and stored in similar conditions. Sera samples were thawed on the same day and IL-2 (Interleukin-2), IL-4 (Interleukin-4), IL-10 (Interleukin-10), IFN-γ (Interferon-gamma) and TGF-β (Transforming growth factor-beta) levels were assessed by ELISA using the commercial kits of MedSystems Diagnostics, Vienna Austria.

STATISTICAL ANALYSIS

Comparison of Hp infected and controls was performed using the Mann-Withney U-test. Analysis of Hp infected patients was performed using the Wilcoxon test. The data is presented as mean ± standard deviation of the mean and p value of <0.05 was accepted as statistically significant.

RESULTS

Percentages of peripheral subtypes of lymphocytes are demonstrated on **Table II.**

Parameter	Before Hp eradication	After Hp eradication	Control group
T lymphocyte CD3 (%) *	47.7±5.1	41.9±4.2	40.6±2.0
T helper CD4 (%)	37.8±6.3	38.1±3.4	36.1±26
T suppressor CD8 (%)	21,8±5.2	25.1±4.7	24.0±2.3
B lymphocyte CD19 (%) **	16.1±3.9	21.9±2.3	22.0±1.9
CD16/56 (%)	8.9±2.6	6.8±2.1	7.2±1.7
CD25(%) ***	14.4±7.5	7.7±1.8	5.9±0.7
CD54(%)	7.7±3.6	6.1±1.6	6.3±0.7

* p<0,001 between study group before Hp eradication and control group; p<0,01 between study group before Hp eradication and after Hp eradication
** p<0,001 between study group before Hp eradication and control group
*** p<0,05 between study group before Hp eradication and control group

Table II: *Percentages of peripheral subtypes of lymphocytes (Values are presented as mean and standard deviation).*

Peripheral T-lymphocyte ratio of Hp positive patients were significantly higher than the Hp negative patients (47.7±5.1% and 40.6±2.0% respectively, p<0.001). After the eradication treatment circulating T-lymphocytes decreased significantly compared to pretreatment levels (41.9±4.2% and 47.7±5.1% respectively, p<0.01). When we compared the Hp eradicated group with the Hp negative group, there was no significant difference in circulating T-lymphocytes between two groups. When we compared lymphocytes according to CD25 positivity; we observed that CD25(+) cells were significantly higher in infected patients than non-infected (14.4±7.5% and 5.9±0.7% respectively, p<0.05). After the eradication of Hp the ratio of CD25(+) lymphocytes decreased, and the statistically significant difference disappeared between the groups. Circulating B-lymphocytes ratio of Hp infected patients before eradication were significantly lower than the controls (16.1±3.9% and 22.0±1.9% respectively, p<0.001). When we compared Hp infected group after eradication with the controls, we observed no significant difference in circulating B-lymphocytes ratio (21.9±2.3, 22.0±1.9% respectively).

As shown on **table III**

Parameter	Before Hp eradication	After Hp eradication	Control group
IFN-γ (pg/ml)	1.2±0.1	1.3±0.4	1.4±0.7
Il-4 (pg/ml)	22.6±20.9	20.6±9.5	30.1±13.2
IL-2 (pg/ml)	1.0±0.2	0.7±0.2	1.2±0.5
IL-10 (pg/ml)	8.5±7.0	11.4±8.7	12.0±8.0
TGF-β (ng/ml)	36.2±5.6	35.4±3.2	33.6±8.0

Table III: *Cytokine levels of patients in Group 1 (before/after Hp eradication) and Group 2 (Values are presented as mean and standard deviation).*

there was no significant difference between the cytokine levels of Group 1 and 2. The cytokine levels did not change after the eradication treatment in Group 1 patients.

DISCUSSION

As mentioned above Hp infection is prevalent in approximetely 50% of the population. However only a small proportion of individuals develop gastritis, peptic ulcer or cancer (1). This may be

due to factors influencing bacterial virulence or the host response to Hp. After being ingested, Hp has to evade bactericidal activity of the gastric luminal contents and enter the mucous layer of the stomach. Hp causes gastric inflammation in infected persons and this inflammatory response occurs as recruitment of neutrophils, T and B lymphocytes, plasma cells and macrophages, and this process causes epithelial cell damage (10). Studies about specific systemic and local immune response have led to conflicting results; Fan et al. demonstrated that CD8 and CD22 positive lamina propria lymphocytes were increased in Hp positive patients. They found that there was a significant ($p<0.05$) increase in CD8+ and CD22+ cells present in lamina propria lymphocytes subpopulations in Hp positive subjects, but no significant difference in the number of CD3+, CD4+, CD14+, and CD56+ cells in Hp positive and negative groups (7). In another study Hatz et al. evaluated the specific subset composition of lymphocytes present in Hp-associated gastritis (HAG). They showed a significant increase in CD4+, CD45RO+, TCR alpha/beta+ activated lamina propria lymphocytes in HAG which correlated with grade and activity of gastritis and degree of bacterial colonisation, whereas subsets of intraepithelial lymphocytes did not change significantly (11). In a more recent study conducted by Agnihotri et al. was shown a decrease in CD4+/CD8+ cells, no change in activated T cells, and an increase in natural killer cells in Hp associated gastritis (12). In different studies it has been demonstrated that gastric epithelium when infected with Hp has enhanced levels of IL-1β, IL-2, IL-6, IL-8 and TNF-α (13-16). Bartchewsky et al implys that since inflammatory response to Hp infection plays an important role in cellular proliferation and gastric mucosal damage, the up-regulation of IL-1beta, IL-8, and COX-2 in patients with chronic gastritis has an important clinical implication in gastric carcinogenesis (17). Another study shows that, there is no systemic alteration in the spesific immune system in response to Hp in patients with duodenal ulcer or chronic antral gastritis compared to Hp negative non-ulcer dyspepsia. In this study, it was shown that there are no alteration in total T and B lymphocytes and $CD4^+$ T, $CD8^+$ T lymphocytes and natural killer cells of both duodenal ulcer and chronic antral gastritis patients compared to normal persons. Although there was a slight increase in the proportion of active T lymphocytes in duodenal ulcer and chronic antral gastritis groups comparing to healthy subjects the difference was not statistically significant (9).

Recent evidence indicates that the secreted Helicobacter pylori vacuolating toxin (VacA) inhibits the activation of T cells. VacA blocks IL-2 secretion in transformed T cell lines. Sundrud et al have published a paper suggesting that VacA of Hp may inhibit the clonal expansion of T cells that have already been activated by Hp antigens, thereby allowing Hp to evade the adaptive immune response and establish chronic infection (18).

We found out that T-lymphocytes were increased due to Hp infection and after eradication T-lymphocyes had returned to the levels of the control subjects. Also it is known that CD25 which functions as IL-2 receptor and activation marker of T lymphocytes, B lymphocytes and monocytes. When we evaluated the expression of CD25 on mononuclear cells we found that CD25 level was increased in Hp infected group compared to control. After eradication treatment CD25+ cells returned to the levels of the controls. In a study published in 2012, authors reported that the percentage of peripheral blood CD4+CD25+ T cells, but not CD4+25+Foxp3+ T cells, increased in Hp-infected patients. Since CD4+CD25+ T cells include regulatory T cells (Tregs) as well as activated T cells, their data indicate that the percentage of Tregs did not increase in the peripheral blood of Hp infected patients (19). We have not been able to detect CD25 positivity on a subset

of lymphocytes but only on cells in lymphocyte gate. We have found similar results with this study by finding increased level of CD25 (IL-2R) in peripheral blood. On the other hand, we could not find any alteration in cytokine levels in Hp infected subjects compared to Hp negative subjects.

CONCLUSION

Our findings indicate that beside local immune response, systemic cellular immune response may also be activated by Hp infection. However, cytokine studies did not support this idea. To conclude a definite decision about the systemic response to Hp infection is difficult due to these conflicting results. Therefore, more comprehensive studies, including more patients with gastric ulcer, duodenal ulcer, gastritis and/or duodenitis, and a wider panel of immunologic markers/cytokines and use of more sensitive methods such as cytometric bead array for detection of cytokines should be performed to clarify this subject.

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